DISSEMINATION OF FIRE BLIGHT

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BULLETIN 357

Tender twigs covered with bags coated with parafin or linseed oil as soon as the buds were swelling in spring to protect them from natural inoculation with blight

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BULLETIN

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DISSEMINATION OF FIRE BLIGHT

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PREFACE

An explanation seems necessary for reporting work which is confessedly incomplete and parts of which need repetition and expansion to obtain satisfactory confirmation. The work is exactly where we were obliged to stop with it in 1917, and most of it where we stopped in early summer, 1916. The resignation of the junior writer and of other members of the staffs of both the Departments of Entomology and Botany have made cooperative effort or indeed any effort at all to resume the work impossible. At present we can see no prospect of being able to resume the project in the future, and to satisfy the inquiries of many entomologists and plant pathologists, we have thought best to publish our somewhat tentative conclusions in the hope that the data submitted will furnish a basis for similar and more complete work by other investigators.

INTRODUCTION

A few years ago the writers began a rather extensive experimental investigation to learn more regarding the inter-relations between the chief factors, both known and unknown, involved in the dissemination of fire blight, the causative organism of which is known to science as *Bacillus amylovorus*.

Many workers were questioning, in print or orally, whether the knowledge of the etiology of this disease was not defective or at least whether a new appraisement was not needed as to the relative importance of the factors known to be concerned.

This attitude of mind was well expressed by Professor D. H. Jones in Bulletin 176, Ontario Agricultural College, pp. 23-24, which read as follows:

*Now with Pennsylvania State College

"The specific cause of the disease once being established the next step is to find out its means of dissemination. Numerous theories concerning the spread of the disease have been promulgated both by scientists and laymen. Mere theories, however, while they may be plausible, are not by any means satisfactory and unless substantiated by direct observation data are not reliable.

"Some observers contend that the wind is responsible for much of the spread. They noticed that the disease progresses through the orchard in the direction of the prevailing winds. They have suggested that the gummy exudate dries on the outside of the tree, powders into dust, and in this condition is blown from tree to tree. While the argument sounds plausible, it has not been verified by experiment. Spores to enable it to tide over unfavorable conditions have not been observed in the organisms under any conditions, and the organism soon dies on drying in the sun, as we have proven both in the laboratory and by outside observations.

"**Blossom Inoculation.**—So far as observed, insects are the principal means of dissemination. Nearly all the twig blight that is noticed in the spring is due to blossoms being infected. Bees, wasps, and other insects visiting the blossoms are said to convey the germ from flower to flower. Waite, of the United States Department of Agriculture, has found the organism in the nectaries of apple blossoms and also on the legs and mouth parts of bees. This evidence is sufficiently conclusive to warrant the statement regarding bees as carriers of the disease.

"The question is, where do the bees in the first place get the contamination? As previously remarked, when the disease is in an active condition either in twig, bark or fruit, a gummy exudate loaded with germs is often found oozing through the epidermis of the affected part. Insects alighting upon or feeding on this material would get contaminated with the germ and carry it away with them attached to their feet and mouth parts particularly. We have found flies, beetles, aphids and other hemiptera feeding on or walking over this area. Though possibly this is how the bee gets contaminated in the first place, the writer has never yet seen one alight on the gummy exudate, and in fact, though careful search was made last season through the College orchard all through blossom time, no gummy exudate could be found on twig, branch or affected trees, and many trees were affected.

"Notwithstanding the fact that no exudate was observed on the trees before or during blossom time, a large number of blossom infections occurred and subsequently developed in various parts of the orchard. If these inoculations were made by bees, from where did the bees get the germ, if there was no gummy exudate for them to come in contact with? We expect to work more on this phase of the subject next season if possible.

"So much, then, for blossom infection. The germ has been found in the nectaries of the flowers and has been found on the proboscis of bees visiting these flowers. But the germ will not develop in the nectaries until it is deposited there. Here our positive knowledge of this phase of the subject ends".

TREND OF OUR INVESTIGATIONS

Since Jones and several other investigators were directing their researches with considerable success toward discovering the part played by sucking insects as carriers of twig blight, we decided to address our efforts more particularly to accounting for the rapid spread of blossom blight. We felt that accepted explanations were inadequate to account for the phenomena observed, and were agreed with several observers in doubting whether local hold-over and exuding cankers could be found in each orchard which suffered to furnish the origin of the sudden and widespread outbreaks sometimes seen; furthermore, we concluded that if pollinating insects were the chief carriers of the organism from blossom to blossom, as was currently believed, then only part of the complexities involved in the transmission and development of the disease was understood and much remained to be discovered.

Since bees had been found by Doctor M. B. Waite* to carry the organism of blight from diseased to healthy blossoms, it became a matter of interest to know whether ripened nectar or honey harbored the organism like raw nectar, thus furnishing a medium through which most or many of the inhabitants of a hive of honey bees could or would become infected without having visited infected blossoms in the orchard. The habit of the honey bee to thrust its mouth into honey in the honey cell when unloading its nectar†, renders contamination of its mouth parts absolutely certain if the honey contained in the cell was stored only a short time before from blighted blossoms, unless nature has supplied some kind of strainer to remove, or some agent to kill, the germs on the way from the blossom to the hive. No such strainer is known, and the presence of any killing agent must be proved by experiments with nectar and honey.

LIFE OF BLIGHT BACILLUS IN HONEY

In 1915 we found that the bacillus would live for 47 hours in honey and published the results in Vol. IX of the Journal of Economic Entomology. In 1916, we investigated further and summed up the results as follows in the Monthly Bulletin of the Ohio Agricultural Experiment Station for September, 1916.

"On May 9, 1916, during the apple-blossoming period, a 5-cubic centimeter sample of honey was taken from the hive by means of a large sterile pipette. This sample, just as it came from the hive, was infected with a 2-day-old culture of the blight organism at 8:40 a. m., May 10. The organism was thoroughly mixed with the honey, which was of a light-colored, watery consistency.

"For 9 days, or from May 11 to May 9 inclusive, inoculations were made from this infected honey into the tips of healthy, tender apple shoots, a sterilized scalpel being used to inoculate the shoots and 200 inoculations and 205

*Eighth Biennial Report California State Board of Horticulture
†ABC and XYZ of Bee Culture by A. I. and E. R Root (Edition 1915) p. 59

checks being made. All were protected with paper bags, which had been placed over the twigs in early spring before the leaves appeared.

"Records taken May 26, 1916, showed that from honey which harbored the organism for 30 hours, 95 8 percent of the inoculated twigs became infected; honey holding the organism 53¼ hours, 33½ percent, and that containing it 72 hours, 28 percent. Every check remained perfectly healthy.

"The maximum life of the organism in honey may exceed 72 hours, but it can hardly be many hours greater than this. From all the evidence we possess, we would be much surprised to find it living longer than 100 hours."

DIRECT INVESTIGATION FOR THE PRESENCE OF BLIGHT IN THE HIVE

We were unable to obtain cultures of fire blight from hives in early spring, from either honey or wax and because of its eventual death in honey, we believe it does not exist in the hive at the opening of the season. Dozens of samples were taken and cultured by the plate method in this quest.

In the spring of 1916 we ran a series of inoculations during blossoming time to learn whether virile blight organisms were present in the honey cells in the hive. Several hundred twigs were protected before and during bloom by being inclosed in paper bags, coated with paraffin or else with oil, to prevent insect visitation. Our honey samples came from three hives located as follows: Two were near the Station orchards, having the usual environments and conditions of hives kept for honey production. The orchard contained nearly all the common fruit trees, apple predominating. Nearby were gardens of small fruits and less than one-half of a mile distant were considerable-sized patches of native woodland and the forestry nurseries of the Experiment Station. Most of the orchards had been in permanent sod for years, so dandelion and other wild spring flowers were very abundant. The honey taken from these hives, therefore, must have been as composite as is gathered into the average hive in the north central states.

Soon after the bloom opened, we commenced taking a honey sample each day in the following manner:

A sterilized pipette was inserted into a honey cell and a few drops of honey were sucked into it, the mouth being applied to the other end of the pipette; a shift was then made to another cell and then to another until a few drops of honey had been taken from each of 25 to 100 cells for each sample. This honey was then expelled by the breath into a sterilized test tube where it was thoroughly stirred by means of a sterilized glass rod, after which it was ready for use. The paper bags were then removed from the twigs chosen for inoculation and incisions were made into the tender growing shoots with a sterilized scalpel. A drop of the honey was then put into the wound with a sterilized instrument and the bag was replaced over the twig and tied in place. The number of inoculations made in this set of experiments was 670.

The third hive was a small one set under a large apple tree that had a frame built over it before the bloom opened and was then completely enclosed in cheesecloth to exclude insect visitors. As soon as this tree was in full blossom the hive was set under it, and 63 of the blossoms, distributed around the circumference of the tree and at different heights, but within reach of a man on the ground or standing on a common stepladder, were inoculated with blight by blowing into them with an atomizer, a water solution of the blight

**Fig. 1.—Inoculated trees with *B. amylovorus* isolated from pure honey after 8 minutes, 1 hour, and 2 hours and 4 minutes incubation in it and then grown on nutrient glucose agar.
Twigs numbered 461-473 inoculated, 100 percent infection.
Check tree free.**

Fig. 2.—Inoculated tree with *B. amylovorus* after incubating in honey for 41 and 43 hours and then grown on 3 percent neutral nutrient glucose agar. Twigs numbered 931-942 inoculated, 100 percent infection. Twigs numbered 943-954, checks, not inoculated, free.

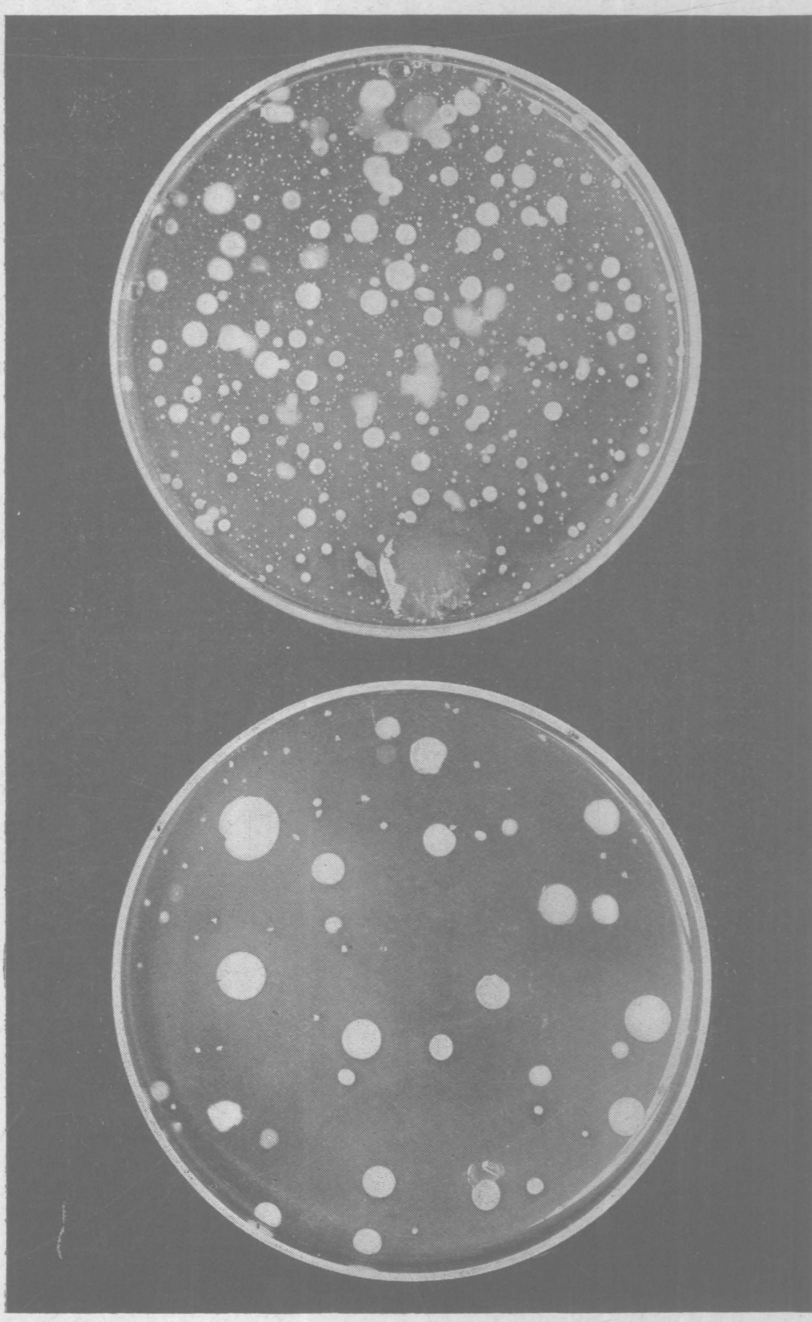


Fig. 3.—Poured plate cultures of *B. amylovorus*. Organism incubated in pure, sterile honey for 5 hours and 37 minutes and then grown on neutral nutrient glucose agar.

organism from a culture 12 days old. Sixty similar inoculations were then made with a water solution made from a culture 1 day old. Then 50 inoculations were made into tender twigs directly from the agar cultures by use of the platinum needle. Most of the inoculations in all these series proved effective, the third series recording 100 percent of blighted twigs. Owing to the agency of rain drip (See page 99), a distinct fringe of blighted blossoms developed around the lower circumference of this tree, but, through the bees, the entire top developed as much or more blight than any other tree in the orchard and the crop of fruit was much reduced. The only source of nectar for the hive under this covered tree was from the apple blossoms, many of which were blighted, and from a few dandelions inside the tent on the ground. We were obliged to supplement the food supply by feeding with sugar syrup. From the honey samples taken from the two hives with natural environment, 473 inoculations in all were made, and from the one under the covered tree, 197. Samples from all three hives were taken and used daily throughout the entire blooming period to insure infection, if distribution of the organism at any time became general in the nectar and survived in the honey.

For so long a time after the work began, all our results were negative, and so little blossom blight was present anywhere in the orchard, that we concluded no results would be forthcoming during this season and relaxed our vigilant watch for blighted twigs in our bags. When, therefore, we found after having omitted examinations for nearly 2 weeks that some twigs were black and dead, we were somewhat handicapped to prove that they had died from blight, though they had every symptom of having done so. Nevertheless, though most of the twigs were too dry to furnish a promising exudate, we obtained it in two instances. From these two twigs cultures were made, examined under the microscope, and used to inoculate some potted apple trees protected from accidental infection by being kept under cheesecloth covers in the insectary. The developments proved beyond question that blight had killed the orchard twigs from which the cultures were taken.

If the conclusion be accepted that all these dead twigs that had apparently died from blight, really did so, then 28 percent died from blight from one series of inoculations from hive No. 1, and 32 percent from another series. From hive No. 2, one series gave a record of 12 percent dying. From hive No. 3, under the covered tree, one series of inoculations made a record of 26 percent of the twigs blighting and another, 48 percent. The organism was recovered from one of these dead twigs, inoculated from hive 3, and its idenity proved by inoculation into a potted apple tree in the greenhouse. During the entire season's work, only one out of several hundred check twigs died with any malady resembling blight, this one, we suppose, being an accidental infection. We have hesitated to publish these data, since it seemed altogether desirable to repeat and confirm them by recovering and proving the identity of the blight organism in every case of apparent infection from the inoculation; but since any prospect for pursuing the inquiry further seems denied to us by force of circumstances, we have thought best to publish the results.

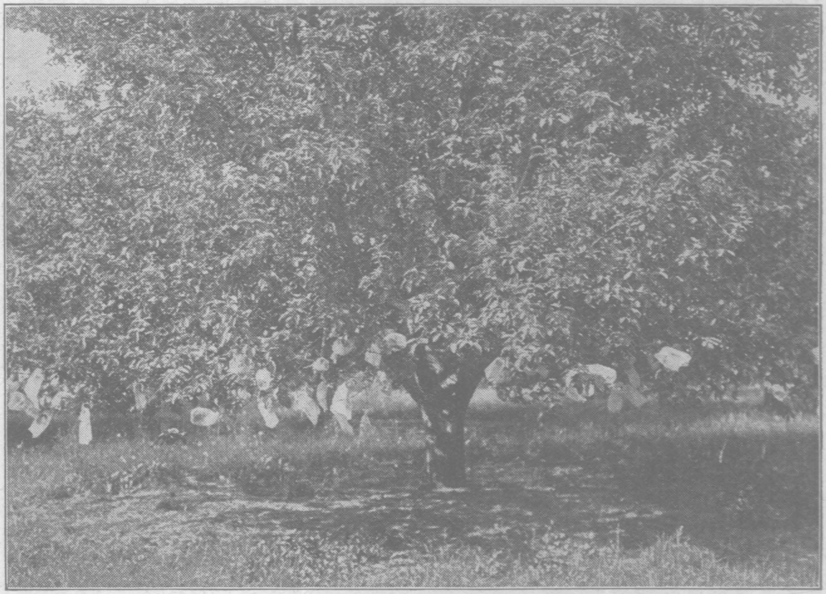


Fig. 4—Tender twigs and blossoms protected against natural infection with fire blight by being covered with paper bags coated over with parafin or linseed oil to exclude insects and rain. Twigs were covered soon after growth commenced, blossoms while in the bud. Covers were replaced after artificial inoculations were made.

The foregoing evidence is much strengthened when considered in connection with that obtained from using pollen from the pollen baskets of bees for inoculating twigs (See page 94), also mouthparts of bees caught at the hive entrance for the same purpose (See page 95) as will be noted subsequently. *All three of these groups of inoculation yielded apparent results from honey samples, pollen and mouthparts, taken on the same date, May 23, and some*

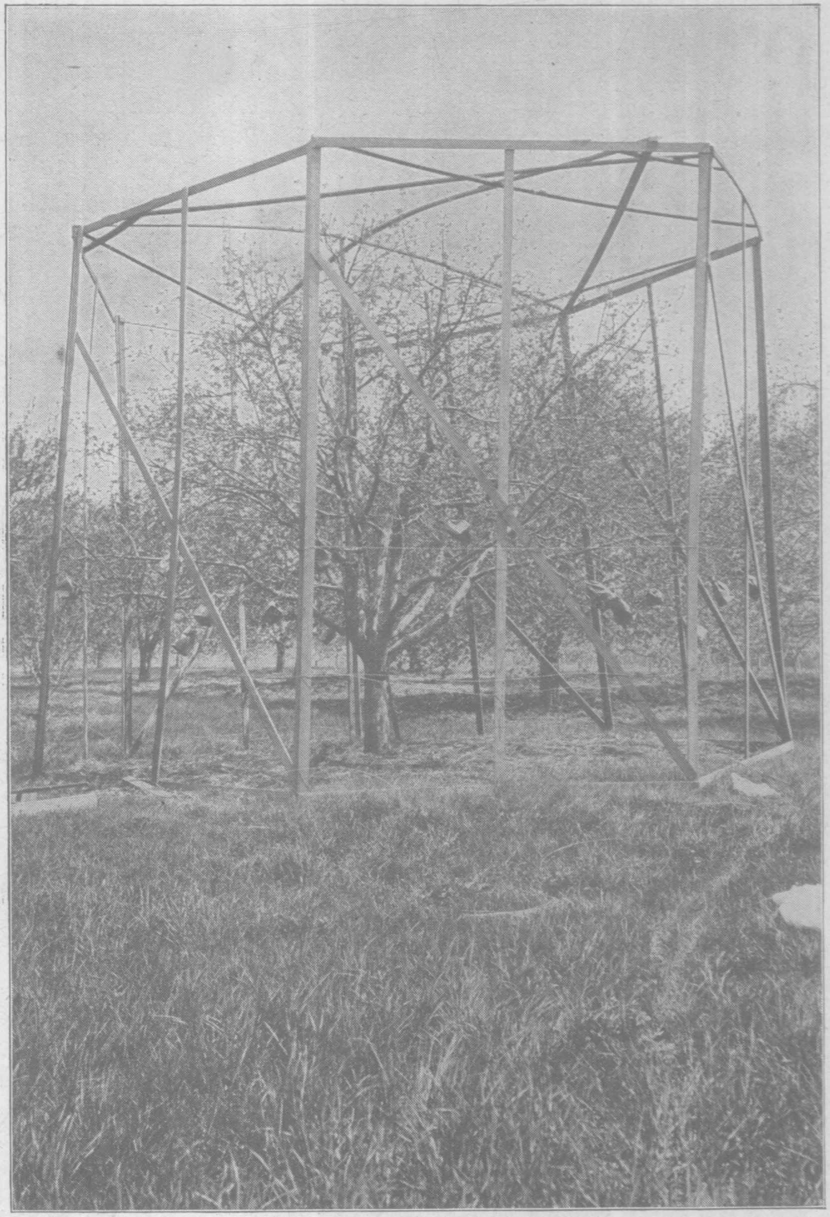


Fig. 5.—Frame to support cheesecloth tent over Wealthy apple tree. Foliage just putting out. Some twigs were bagged at an earlier date before the tree was chosen for tenting

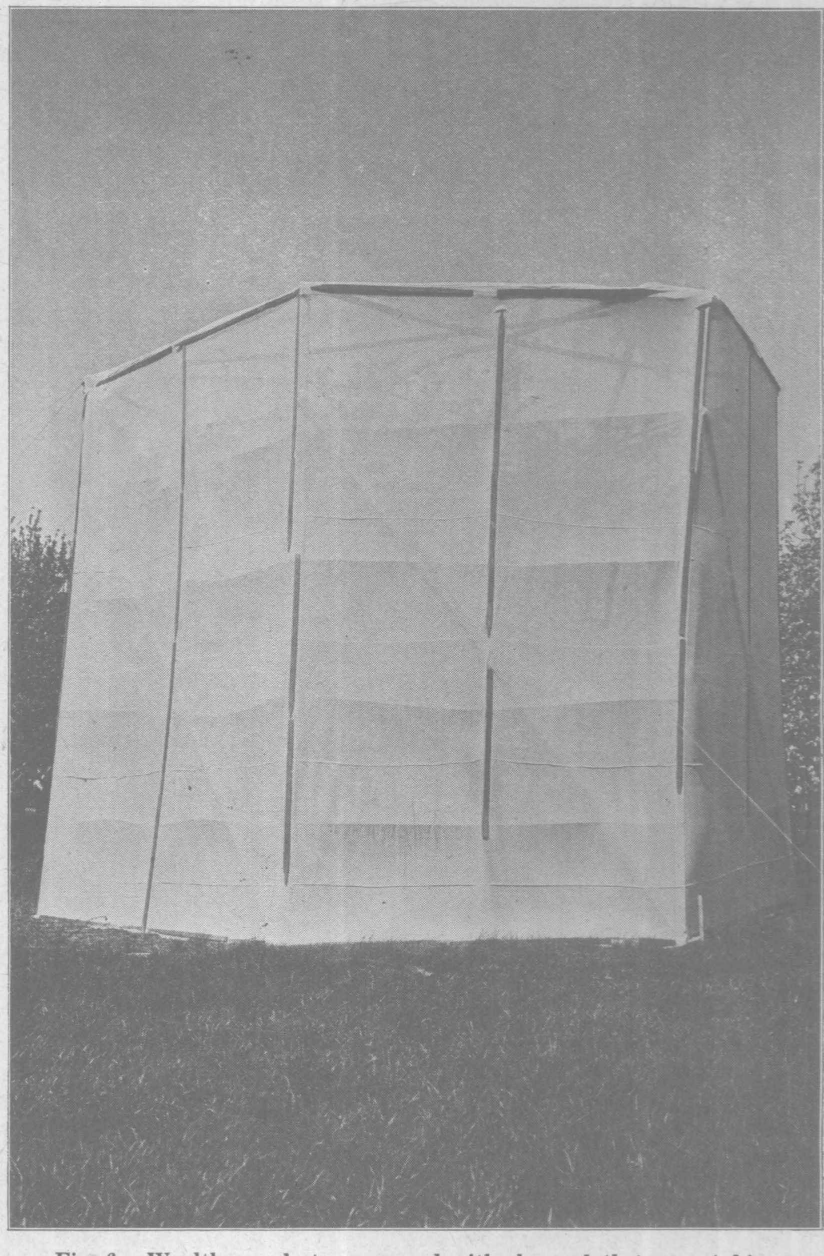


Fig. 6.—Wealthy apple tree covered with cheesecloth tent. A hive of bees were confined in this tent

additional results with mouthparts taken May 24. The weather conditions prevailing at this period were such as to favor the developments recorded. May 18 had a mean temperature of 45 degrees and was cloudy, discouraging the work of bees. May 19 had a light frost and a mean temperature of 50 degrees, but was clear. May 20 had a light frost, recorded a mean temperature of 49½ degrees and was one-half clear. May 21 was warmer, mean temperature 56 degrees and three-fourths clear. May 22 was warmer, mean temperature 50 degrees, cloudy and, of great significance, had .47 inches of rain, thereby scattering broadcast over the blossoms the blight organism from such centers as were then established. May 23, the temperature rose to a mean of 69 degrees and the day was clear, favoring great activity on the part of the bees. May 24 was somewhat of a repetition of the preceding day, the mean temperature being 67 degrees and the day was clear. These are the notes kept by the Station weather observer and were recorded without reference to our problem; in fact, we first obtained them December 18, 1919. It is also worthy of note that the first indication of general blighting among the blossoms was seen May 23, and that our notes record under date of May 24 that our covered tree was "quite generally blighted."

We believe there is much warrant for concluding that blight centers had been established by insects prior to May 23, but that most of the blossoms remained free of the contagion until the rain of May 22 gave it wide distribution and the rising temperature enabled the organism to multiply rapidly wherever it lodged in the nectar. Consequently, honey in the hive, pollen in the bees' baskets, and the mouthparts of the bees all became more or less contaminated on the 23 and 24 of May.

DOES BLIGHT ENTER THE HIVE WITH POLLEN?

To determine this point we caught bees laden with pollen in a net as they were entering the hive, some of which were killed in cyanide bottles. We had previously ascertained that blight germs were not destroyed by exposures in the cyanide bottle during the interval necessary to kill a bee; but most of the bees were killed by crushing the thorax with sterilized forceps or by a pinch with the fingers, keeping a thickness of the bag cloth between the fingers and the insect. The pollen was removed from the baskets by sterilized tools and small pieces of it were inserted into wounds made into tender twigs with a sterilized scalpel. The number of inoculations made was 168 on seven different dates, with an interval of 2 days between two of the series of inoculations, all others being on successive days. Only one, that of May 23, gave apparent results. Records were not taken until 3 or 4 weeks after the inoculations were made. Six twigs of one series, distributed in three different bags of heavy brown paper, were black and dead, looking as if they had died from blight; but there were no exudation drops and it was impossible to recover and prove the identity of the organism. Some healthy green twigs also were found in each of the bags, quite certainly indicating that the dead twigs did not die from being scorched by heat. There was but little blight outside the bags on the tree on which the twigs were found. As a matter of course, this evidence is not reliable and cannot be accepted, unless confirmed by repetition and recovery of the undoubted organism of blight; but a strong and significant point, favoring the conclusion that genuine blight developed, lies in the fact that this series of inoculations was made May 23, the date that yielded results with honey inoculations from three different hives and also was but 1 day before the date that gave the most decisive results from inoculations made with the mouthparts of bees as recorded in the next section.

IS BLIGHT CARRIED ON MOUTHPARTS OF BEES?

To determine this question we made a series of inoculations as follows:

Bees were captured at the hive entrance as they were entering or leaving and were killed by pinching the thorax, keeping one or more thicknesses of the cheesecloth net between the fingers and the insect. They were then put into a sterilized bottle and taken immediately to apple trees having fresh tender twigs which had been covered by paraffined or oiled paper bags for a considerable time previous to blooming. Here the mouthparts were torn away with sterile forceps and inserted into wounds made with sterile instruments, in the selected twigs, after which the protecting paper bags were again put in place. Nine of these inoculations were made before bloom and two sterile incisions were made into other twigs to serve as checks. Just as we expected, no blight, nor anything suggesting it, ever developed from these pre-blossom inoculations nor on the checks.

After the trees were well in bloom, we captured bees in the manner before described and made similar inoculations each day for 19 days, beginning May 8. In all, 205 inoculations were made from the same number of bees, and 56 twigs into which sterile incisions had been made were kept as checks. If 299 checks kept for other series be included, 355 checks which remained free from everything resembling blight were available for comparison.

In the series made May 12, all were recorded as free from blight, though two were regarded as doubtful.

Everything else remained free from suspicion until we reached the series made May 23, when two twigs inoculated from bees which had been captured from hive No. 2 were found dead; also several inoculated May 24 from bees captured from the hive under the covered tree were dead. Altogether 30 percent of the inoculated twigs on this one tree had died, apparently from blight. There was no virile exudate, the examinations having been delayed until 3 or 4 weeks after inoculation, so it was impossible to recover and prove the identity of the organism. No blighted twigs were to be found on the tree unless in the bags. In another series made the same day from the hive under the covered tree, 30 percent of the twigs died in three different bags, each of which also contained a healthy living twig. The junior writer recorded that there was little doubt that the twigs in two of the three bags had died from blight, though the twigs were too dry to permit the recovery of the organism. In a series of inoculations made May 24, with bees taken from hive No. 2, 10 percent of the twigs were dead on June 2. The junior writer who made the examination was in no doubt about two of the twigs having died from blight. There was very little blight on the tree, but these two twigs were in a cheesecloth bag which would not exclude the possibility of infection by raindrip which might exonerate the bees. From a series of inoculations made on May 25 with bees from the covered tree, 20 percent of the twigs died, apparently from blight. The weak point in the proof based on these results lies in the fact that the organism could not be recovered and its identity proved; the strong point that none of the checks died in similar manner, and that nearly all of them (the exceptions have been noted above) were in paraffined or oiled paper bags, thus excluding the possibility of infection by rain, wind, or insects; also corroborative evidence lies in the fact that these apparent results came from inoculations made May 23, 24 and 25, the same dates on which we obtained apparent results from inoculations made with honey from three different hives and from pollen taken from the legs of bees. It should be noted that the organism was recovered and proved from several of the inoculations just mentioned.

The results on the tree covered with cheesecloth gave strong confirmation that the bees carried the infection. The inoculations were made, as previously stated, in a broad band around the lower circumference of the tree. Infection eventually spread over the entire tree, this being one of the worst-blighted trees we have ever seen; but the original broad band of inoculation and the dead flowers below stood conspicuously out as the worst-blighted portion. After we proved in the following year that raindrip was a great factor in spreading infection, this phenomenon became readily intelligible. A tree of the same variety, Wealthy, standing next in the row to the covered tree, developed very little blight until we showed some mercy to the imprisoned bees near the end of the blooming period and moved them under it. Then both it and another adjacent Wealthy tree developed severe blight infection in about 10 days time. The following year when we covered a tree with cheesecloth before bloom to exclude all insects and made no inoculations on it, not a sign of blight developed. Of course other insects than bees were present on this covered tree, used in 1916, but they were not more numerous nor of different kind than those on the adjacent uncovered trees of the same variety.

Fig. 7.—Protecting twigs and blossoms from natural infection by covering them early in the season with waxed or oiled paper bags to exclude insects and rain. More than 1,000 of these were used in the spring of 1916.

DOES THE BACILLUS LIVE IN APHID HONEY-DEW?

From the fact that aphid honey-dew is visited by great numbers of flies, ants, wasps and bees of various kinds, its capacity for sustaining the life of the bacilli is necessarily of much interest. We had excellent material with which to work in the Station entomological and botanical greenhouses. This consisted of young potted apple trees badly infested with the apple aphis, *Rhopalosiphum prunifolium.*

Two leaves were selected for this study. On the upper side of one, which was not curled but in a horizontal position, was a large drop of honey-dew at the basal end. This honey-dew came from aphids on a tender, everhanging shoot directly above. On the under side of the other, which was curled, were two large drops, and these were continually added to by the presence of aphids within the curled leaf above the drops. Honey-dew was collected in small quantity in a 1-cubic-centimeter pipette from trees close by and to the drops on the two leaves was added sufficient honey-dew to form one large drop on each leaf.

The two leaves were then separately infected with a 9-day-old culture of the fire-blight organism, one at 2:30 p. m. and the other at 2:55 p. m. on April 24, 1916. Each was inclosed in a paper bag to prevent rapid evaporation of the drops. Check inoculations were then made into tender apple tips with this same culture in order to prove beyond question its identity. These inoculated shoots subsequently became infected, while sterile incision checks made at the same time remained free. Sterile incision checks were made with a sterile scalpel into tips similar to those used for inoculation.

Every day for 10 days, or until May 4, inoculations were made from the infected honey-dew drops into healthy apple shoots, and check inoculations consisting of sterile incisions were made at the same time.

The honey-dew drops slowly evaporated until at the end of 5 days they were only moist in the mornings and dry the remainder of the day. The seventh day showed them to be completely dry. A small quantity of sterile water (two or three small drops) was then added each day, after the seventh, to the place where the honey-dew had been, this being kept up until the end of the tenth day. Inoculations were also continued from the water added to the dried honey-dew until the end of the tenth day.

Results taken on May 19 showed 100 percent infection of all inoculations with the infected honey-dew, while all checks remained free. In other words, the blight organism can live in honey-dew for 7 full days until the dew has completely dried, and then for 3 more days, or perhaps longer, when moisture is added.

WILL THE BLIGHT ORGANISM SURVIVE IN PEACH, PLUM AND CHERRY BLOSSOMS?

We have thus far experimented only with peach, plum and cherry blossoms in connection with this question. It is desirable, of course, to conduct similar tests with many or all of the blossoms which are open during the same period as blossoms of the apple, pear and quince.

A water suspension of the blight organism was made by washing a 2-day-old culture of the organism with sterile distilled water into a 100-cubic-centimeter flask. Inoculations were made on May 9 and 10 by means of a camel's-hair brush into 13 peach (Connet's Early and Arp Beauty), 16 plum (Missouri apricot) and 11 cherry (Montmorency) blossoms, the water suspension being used as the inoculum.

Approximately every 24 hours thereafter, specimens of these inoculated whole blossoms were taken, the petals cut off with sterile scissors and the calyx cups cut into small parts into a sterile receptacle. These parts were then used as the inoculum in inoculating tender apple shoots. The test extended over 5 days, and altogether 85 inoculations were made from the peach and 45 each from plum and cherry. Practically as many checks were made as inoculations.

Records taken on May 26 showed an average of 64½ percent infections from the peach blossoms, 74 percent infection from the plum and 94 percent from the cherry. The checks consisting of sterile incisions, all remained free.

These results mean that the blight organism is capable of living in peach, plum and cherry nectar for 5 days or probably more, and during this period can be disseminated from these blossoms to hosts susceptible to blight infection, provided the bacilli have been left there by pollinating insects or other agents.

RAIN AN IMPORTANT CARRIER

As early as 1913 the senior writer noted phenomena suggesting that rain is an important carrier of fire blight infection. Certain twigs of apple which, before bloom, had been inclosed in bags of cheesecloth to prevent insect visitation to them, to be used later for various experiments with aphids, honey bees, bumblebees, wild bees, etc., developed blight both in the check bags, into which no infected insects were introduced, and in those inside which contaminated insects had been loosed. Thus were all our proofs establishing a case against these insects as carriers of blight invalidated, for the experiments had been made in the open orchard. In some of the check bags aphids and similar insects were found, having hatched from eggs that were concealed on or in the twigs at the time when they were covered, but in others scarcely any insects at all could be found; yet all the twigs had been selected because of their healthful appearance, making it quite unbelievable that any blight cankers had been inclosed when the bags were put over them.

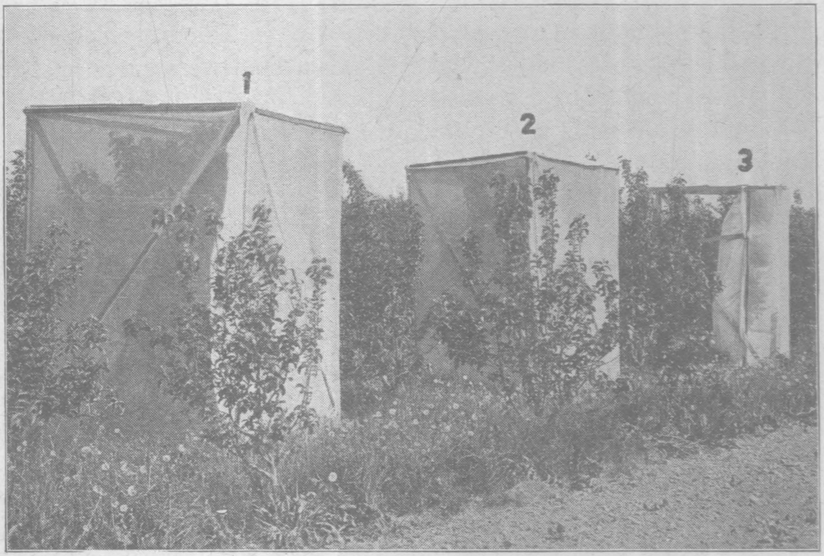


Fig. 8.—Method of covering trees to ascertain function of rain as a disseminator of fire blight

The season of 1914 gave a repetition of the phenomena of 1913. Blighted twigs were found within some cheesecloth bags on June 6, which had been unopened since they were put in place when the bloom buds were first beginning to show pink. The examinations were made, accompanied by Prof. A. D. Selby, who entered this note: "In bags where blighted twigs occurred, some had blighted branches above them, so infection was possible," meaning from raindrip, which the senior writer had proposed as an explanation for the infection.

Beginning with the season of 1915, the writers, respectively from the Departments of Entomology and of Botany of the Ohio

Agricultural Experiment Station, worked conjointly on the various problems connected with the spread of this disease. Cheesecloth bags were put over many twigs and small limbs on April 26 to prevent natural infection by insect carriers, and 41 of these were kept unopened until July 13. Of these 41 checks, 26 were found free from blight, 11 were slightly blighted at the tip and 4 were blighted at other places. The bags were all intact and without openings through which insects could pass. In nearly every case where such blighted check twigs were found, blighted twigs were plentiful above them, and in every case rain could have been dashed first over the blighted twigs from the side if not from above.

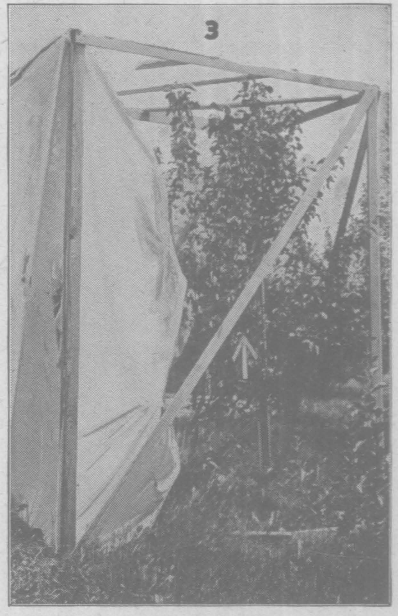


Fig. 9.—An enlargement of Tree No. 3 in Fig. 8

The same phenomena were noted again in 1916, particularly in case of three large bags, approximately 8 feet long by 4 feet wide, used to inclose branches of considerable size for a pollination experiment. On May 11, a late-blooming tree was chosen for the experiment, and the few blooms that were sufficiently opened to render pollination at all possible were removed and only unopened buds left on the branches to be bagged. On one of these branches were 177 bloom-buds; on the second 240, and on the third 228. These branches and the entire tree were apparently wholly free from blight on May 11. All these bags were intact June 26, and insects could at no time have passed through them; yet there was just as much of the disease inside them as outside, of both blossom blight and twig blight. The question entered in our notes was this; "Was blight carried into the bags by rain, which then entered the tissues through punctures made by sucking insects? Was a water solution of the disease washed into the naked nectaries, or had it been scattered in honey-dew, or else inoculated into the tissues by sucking insects before the bags were put in place?" It was also remembered that infection was not absolutely precluded when pinching off the more advanced blossoms, but no blight was at that time evident among the blossoms anywhere in the orchard. Hence, infection from the fingers was regarded as highly improbable.

Plan of experiments.—To obtain a more definite answer to these questions, the experiments of 1917 were planned.

Three 8-year-old apple trees (Duchess of Oldenburg) standing about 12 feet high, were selected for inclosure, the trees marked 1 and 3 of Figure 8 (The tree shown as 3 in Figs. 9 and 10 is the same as 3 in Fig. 8) being used for the rain-infection experiment. Around each of these was built a framework as shown in the illustration, and on May 12, tree No. 1 was inclosed with cheesecloth as shown in Figure 8, no opening being left through which pollinating insects could possibly enter. Not a single blossom was open nor had any opened when the tree was covered. This was kept for a check.

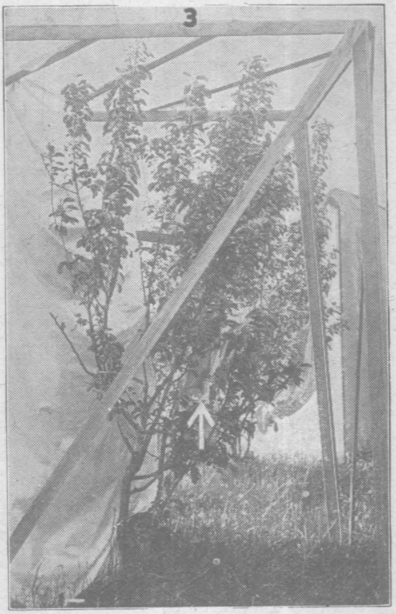


Fig. 10.—Same as Fig. 9 before removal of cheesecloth bags as indicated by arrow

Only one-half of tree No. 3 was covered (May 12), the west wall of the covering being cheesecloth and the top, south, east and north walls being oilcloth. The whole east half of the tree was left uncovered, with the exception of some branches in cheesecloth bags as described later. This tree naturally yielded itself by its habit of growth to this vertical partitioning, and the oilcloth was so adjusted that no rainwater could dash from any part of the uncovered section upon the covered west half. Since our dashing spring rains generally come from a westerly quarter, we chose to leave the east half for the uncovered part. Each half of the tree now had plenty of light and air, but the west half was shielded from blight infection, either through insect visitation or from rain which could first dash over blighted branches or blossoms and then over the protected ones; while the east half was subject to both. To protect part of the east half from infection by insects, four large

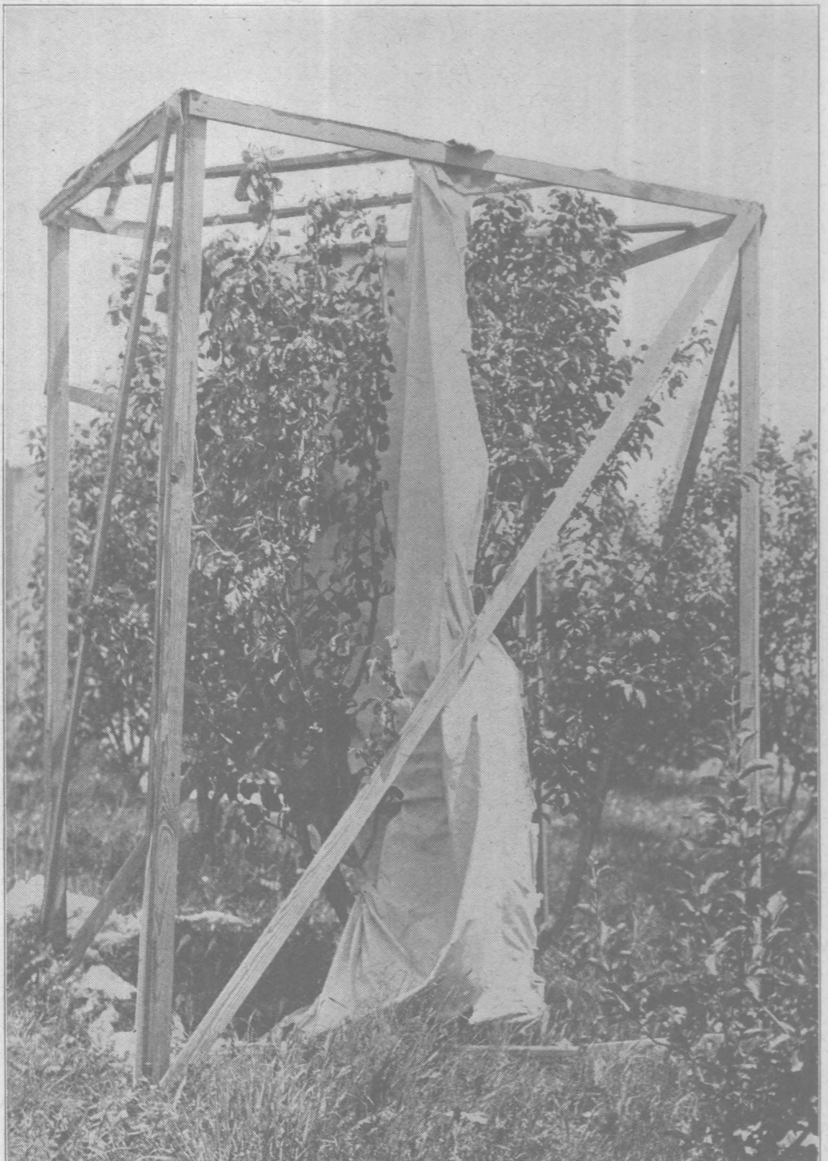


Fig. 11.—Oilcloth partition ready to remove from between the 2 halves of tree numbered 3 in Figs. 8, 9 and 10

cheesecloth bags were used to inclose branches 1 to 2 feet long, bearing unopened blossom buds near the bottom of the tree, beneath overhanging blossom buds. (See the arrow in Fig. 10. The same method is better illustrated in Fig. 13). Thus the tree was divided into three regions: (1) One exposed to natural infection of every sort, including insect visitation and rain entrance; (2) one barred from insect inoculation but exposed to rain infection; and (3) one with the entire west half of the tree barred from infection by both insects and rain. No blossoms had opened, nor was there any sign of blight on the tree when it was covered.

Two days after the trees were inclosed, the central buds of many of the blossom clusters were open. Those blossoms located above the cheesecloth bags, Figure 10, were artificially inoculated with a water suspension of the fire-blight organism, *Bacillus amylovorus* Burrill. This suspension was made by using a pure culture of the organism. Check twig inoculations made the same day proved the virulency of the organism by later withering the twigs with blight.

The method of making the inoculations varied. Some were made to parallel the supposed manner of rain infection by spraying the blight solution into the calyx cup and over the nectaries with an atomizer without making wounds; some were made in a similar manner after making incisions in the interior of the blossoms with a scalpel, and others by introducing the bristles of a camel's-hair brush, wet with the inoculum, into the calyx cup.

Results of experiments.—The inoculated blossoms above the bags were turning brown by May 24, and on May 31 blighted blossoms were found inside the cheesecloth bags, which had not been opened since the date they were put on (May 12).

Originally (May 14) only the central blossom of each cluster was artificially inoculated, whereas 17 days later infection was quite general over the uncovered half of the tree, both inside and outside the cheesecloth bags. (Fig. 12 illustrates infected twigs and leaves on tree No. 3, taken at the point indicated by the arrow shown in Fig. 9).

The check tree, No. 1 (See Fig. 8) was absolutely free from blight on this date (May 31) as was that portion of tree No. 3 (Figs. 9, 10 and 11) which was inclosed in oilcloth and cheesecloth combined.

The experiment of bagging branches was carried out in duplicate on an Oldenburg tree nearby (Fig. 13), the work being done on the same dates as those given above. Seven branches contain-

Fig. 12.—A severely blighted area taken at the point indicated by the arrow in Fig. 9. No marked difference can be seen in fire blight infection between uncovered branches and those covered by cheesecloth bags

ing unopened blossom buds were inclosed May 12, and 2 days later inoculations were made in open blossoms above the bags. On May 31, 17 days after the inoculations were made, the blossoms within the bags as well as those above were nearly all infected.

With the exception of temperature, which was abnormally cool, the weather conditions were favorable for the development of the experiment. Throughout the incubation period of the blight organism in the blossoms, rainfall was sufficiently plentiful to serve the purpose of the experiment. For 3 days after the inoculations were made there was no precipitation whatever, but for the next 6 days, or until May 23 inclusive, showers of rain fell every day, the total precipitation for the 6 days amounting to 1.1 inches, with sufficient wind to scatter the raindrip broadcast. From the 24th until the end of the month, 1.51 inches fell on 4 separate days. It seems reasonable to believe that dissemination occurred during the 6 successive days when showers of rain fell, or from the 18th to the 23d inclusive. Since the inoculations were made May 14, the bacilli would most certainly be present in countless numbers in the nectar of the blossoms by the 18th, when the first rain occurred. As mentioned above, infection was

Fig. 13.—Cheesecloth bags protect branches from blight infection by insects but not by raindrip

not noticed within the bags until May 31; consequently there was an interval of 13 days in which infection took place before becoming noticeable.

Other experiments with fire blight conducted during the spring of 1917 tend to confirm the foregoing evidence. Hundreds of inoculations were made in blossoms, and it was always noticeable that after a week to 10 days, infection became quite general among blossoms in the vicinity of the inoculated ones. Proof in cases of this kind is, of course, by no means complete or absolute, because pollinating insects may have been partly instrumental, but in the light of the evidence just advanced, it appears quite certain that much of this infection was due to raindrip. Had insects been important agents of dissemination, the infection ought to have been more general and ascended higher into the trees. A broad band of blighted bloom was usually conspicuous around the lower circumference of the trees, evidently being dependent in considerable measure for its shape upon the circle of blossoms which had been artificially inoculated.

One other case gave contributing evidence of rain as a carrier of fire-blight. A large King apple tree was badly infected with both blossom and twig blight, and an adjoining Grimes tree was also infected but only on the side next to the King. The diseased parts of the Grimes tree were in close proximity to those of the King, but somewhat lower, so that it was easily possible that raindrip could have blown from the King to the Grimes. This also is, of course, not proof, but merely contributing evidence. Of course, raindrip does not become an agent of dissemination until primary centers of infection have been established, in nearly all cases, by insects. We have already noticed that aphid honey-dew will support the life of the blight organism for at least 10 days (See p. 98 and also pp. 274-276, Ohio Agr. Exp. Sta. Mo. Bul. Vol. 1 (1916), No. 9). If this contaminated honey-dew is washed over branches beneath, especially over branches harboring other colonies of aphids or similar sucking insects, such as red bugs, infection and spread of the disease would quite certainly follow.

We have become satisfied that from 50 to 90 percent, and sometimes more, of all blossom infection is due to infected raindrip and not to contaminated insects as hitherto supposed. This explains the rapid spread of blossom blight after the primary centers of infection have been established by insects, especially where such centers are located well toward the tops of the trees, thus exposing the hundreds of blossoms below to a bath of infected water. However, the possibility of contaminated pollen or of dried particles of honey-dew, dried exudate, etc. carrying it, is not excluded, and we hope that Professors Stevens, Ruth and Spooner of the University of Illinois, who announced in Science, Vol. 48, p. 449, that the disease is windborne, will discover for us what particles, if any, other than water may serve as a means of transportation through the air. Our announcement that rain furnished one such means of transport preceded their announcement by several months.

EFFECT OF POLLINATION ON SUSCEPTIBILITY TO BLIGHT

Early in the investigation we recognized that the relation of bees to spread of blight might hinge wholly on the effect of pollination. If a fertilized blossom was less susceptible to blight than an unfertilized one, the bees, by effecting early pollination might, on the whole, be restricting the development of blight, even while many of them were scattering it. Such a result of their work seemed to us not at all unlikely and would be in substantial accord with the largest and best body of opinion of both orchardists and beekeepers, who think they experience no more blight and have better fruit crops when bees are present than when they are absent. To determine, if possible, the truth of this relation we investigated as follows:

A large number of blossom buds ready to open within a few hours were chosen and emasculated with sterile implements. These blossoms were then immediately inclosed in paper bags, one bud to a bag, to prevent natural pollination. The next day a series of these were pollinated and a record kept of the date. A few hours later, some of the pollinated blossoms were inoculated with blight; then on each day for from 5 to 11 successive days this operation was repeated with other series, and kept up till the close of the blooming period. These operations were intended to discover how long a pistil could wait after being ripe to receive pollen and set fruit, and also whether susceptibility to blight depended in any way on the lapse of time between pollination and inoculation.

The following tables show the results of the inquiry:

INFLUENCE OF POLLINATION ON SUSCEPTIBILITY TO BLIGHT I

98 Blossoms emasculated May 16, 1917

Series	Date pollinated	Date inoculated	Interval between pollination and inoculation	Inoculated	Blighted	Fruits set	Lost	Blighted	Fruits set	Notes
			Hours	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*	
A	May 17, 1917	May 17, 1917	5	3	3	0	0	100	0	
	May 17, 1917	May 18, 1917	24	3	3	0	0	100	0	
	May 17, 1917	May 19, 1917	49	3	3	0	0	100	0	
	May 17, 1917	May 20, 1917	72	3	0	3	0	0	100	
	May 17, 1917	May 21, 1917	96	3	0	3	0	0	100	
B	May 18, 1917	May 19, 1917	19	2	2	0	0	100	0	
	May 18, 1917	May 20, 1917	48	2	0	0	....	0	0	
	May 18, 1917	May 21, 1917	70	2	0	1	1	0	50	
	May 18, 1917	May 22, 1917	96	2	0	1	1	0	50	
	May 18, 1917	May 23, 1917	120	....	....	....	1	....	....	
C	May 19, 1917	May 20, 1917	24	3	0	1	1	0	33	Diseased blossom clusters appearing around bags due to spray from atomizer and to rain.
	May 19, 1917	May 21, 1917	49	3	0	1	1	0	33	
	May 19, 1917	May 22, 1917	72	3	0	1	1	0	33	
	May 19, 1917	May 23, 1917	96	3	0	2	....	0	66	
	May 19, 1917	May 24, 1917	120	3	0	0	1	0	0	
D	15 blossoms pollinated May 20, 1917 on 6 successive days. No set of fruit, no blight but one blossom suspicious. Petals fallen.									
E	10 blossoms pollinated May 21, 1917 on 6 successive days. No set of fruit, no blight but one blossom suspicious. Petals fallen.									
F	10 blossoms pollinated May 22, 1917 on 6 successive days. No set of fruit, no blight but one blossom suspicious. Petals fallen.									
G	10 blossoms pollinated May 23, 1917 on 6 successive days. No set of fruit, no blight but one blossom suspicious. Petals fallen.									
H	3 blossoms pollinated May 24, 1917 on 6 successive days. No set of fruit, no blight but one blossom suspicious. Petals fallen.									

Check inoculations in blossoms and twigs proved the organism virulent in every case.

INFLUENCE OF POLLINATION ON SUSCEPTIBILITY TO BLIGHT II

301 blossoms about ready to open emasculated on three trees May 18, 1917

Series	Date pollinated	Date inoculated	Interval between pollination and inoculation	Inoculated	Blighted	Fruits set	Lost	Blighted	Fruits set	Notes
			Hours	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*	
I	May 19, 1917	May 20, 1917	26	3	2	1	1	67	33	
Tree 244	May 19, 1917	May 21, 1917	48	3	1	1	1	33	33	
Petals persisting	May 19, 1917	May 22, 1917	76	3	2	0	1	67	0	
	May 19, 1917	May 23, 1917	96	3	0	2	1	0	67	
	May 19, 1917	May 24, 1917	120	3	0	3	0	0	100	
	May 19, 1917	May 25, 1917	144	3	1	1	1	33	33	
	May 19, 1917	May 26, 1917	168	3	0	1	2	0	33	2 apples to size of big pea, then dropped.
	May 19, 1917	May 27, 1917	192	3	0	1	2	0	33	
	May 19, 1917	May 28, 1917	216	3	0	2	1	0	67	
	May 19, 1917	May 29, 1917	240	3	0	2	1	0	67	
	May 19, 1917	May 30, 1917	264	1	0	0	1	0	0	
J	May 20, 1917	May 21, 1917	24	3	3	0	0	100	0	
Tree 244	May 20, 1917	May 22, 1917	48	3	2	1	0	67	33	
Petals persisting	May 20, 1917	May 23, 1917	72	3	1	1	1	33	33	
	May 20, 1917	May 24, 1917	96	3	0	3	0	0	100	
	May 20, 1917	May 25, 1917	120	3	0	3	0	0	100	
	May 20, 1917	May 26, 1917	144	3	0	3	0	0	100	
	May 20, 1917	May 27, 1917	168	3	0	1	2	0	33	
	May 20, 1917	May 28, 1917	192	3	0	3	0	0	100	
	May 20, 1917	May 29, 1917	216	2	0	1	1	0	50	
K	May 21, 1917	May 22, 1917	24	3	2	....	Eaten off	67	....	
Tree 244	May 21, 1917	May 23, 1917	48	3	0	3	0	0	100	
Petals dropping	May 21, 1917	May 24, 1917	72	3	1	1	1	33	33	
	May 21, 1917	May 25, 1917	96	3	1	2	0	33	67	
	May 21, 1917	May 26, 1917	120	3	0	3	0	0	100	
	May 21, 1917	May 27, 1917	144	3	1	1	1	33	33	
	May 21, 1917	May 28, 1917	168	3	0	1	2	0	67	
	May 21, 1917	May 29, 1917	192	3	0	3	0	0	100	
	May 21, 1917	May 30, 1917	216	3	0	2	1	0	67	
	May 21, 1917	May 31, 1917	240	3	0	2	1	0	67	

INFLUENCE OF POLLINATION ON SUSCEPTIBILITY TO BLIGHT III

Series	Date pollinated	Date inoculated	Interval between pollination and inoculation	Inoculated	Blighted	Fruits set	Lost	Blighted	Fruits set
			Hours	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*
L	May 22, 1917	May 23, 1917	24	3	1	1	1	33	33
	May 22, 1917	May 24, 1917	48	3	1	2	0	33	67
	May 22, 1917	May 25, 1917	72	3	1	2	0	33	67
	May 22, 1917	May 26, 1917	96	3	0	2	1	0	67
	May 22, 1917	May 27, 1917	120	3	0	2	1	0	67
	May 22, 1917	May 28, 1917	144	3	0	3	0	0	100
	May 22, 1917	May 29, 1917	168	3	0	2	Broken	0	67
	May 22, 1917	May 30. 1917	192	3	0	2	1	0	67
	May 22, 1917	May 31, 1917	216	2	0	2	0	0	100
M	May 23, 1917	May 24, 1917	24	3	1	0	2	33	0
	May 23, 1917	May 25, 1917	48	3	0	1	2	0	33
	May 23, 1917	May 26, 1917	72	3	0	2	1	0	67
Tree 243	May 23, 1917	May 27, 1917	96	3	0	2	1	0	67
Stems of blossoms yellow,	May 23, 1917	May 28, 1917	120	3	0	1	2	0	33
many pistils brown	May 23, 1917	May 29, 1917	144	3	0	1	2	Fruit suspicious	
	May 23, 1917	May 30, 1917	168	3	0	2	1	0	67
	May 23, 1917	June 1, 1917	192	3	0	0	3	0	0
N	May 24, 1917	May 25, 1917	24	3	0	0	3	0	0
	May 24, 1917	May 26, 1917	48	3	0	0	3	0	0
	May 24, 1917	May 27, 1917	72	3	0	1	2	0	33
Tree 243	May 24, 1917	May 28, 1917	96	3	0	0	3	0	0
Pistils slightly brown	May 24, 1917	May 29, 1917	120	3	0	0	3	0	0
	May 24, 1917	May 30, 1917	144	3	0	1	2	0	33
	May 24, 1917	May 31, 1917	168	3	0	0	3	0	0
	May 24, 1917	June 1, 1917	192	3	0	1	2	0	0
O	May 25, 1917	May 26, 1917	24	3	0	0	3	0	0
	May 25, 1917	May 27, 1917	48	3	0	0	3	0	0
	May 25, 1917	May 28, 1917	72	3	0	1	2	0	33
Tree 243	May 25, 1917	May 29, 1917	96	3	0	0	3	0	0
Pistils slightly brown	May 25, 1917	May 30, 1917	120	3	0	1	2	0	33
	May 25, 1917	May 31, 1917	144	3	0	0	3	0	0
	May 25, 1917	June 1, 1917	168	3	0	0	3	0	0

Series P and Q yielded not more than two set fruits in all and one of these doubtful. No blight in any instance.

Directly connected with this inquiry is the question whether nectar production ceases sooner in case of pollinated than unpollinated blossoms. If nectar is secreted less copiously or not at all soon after pollination has been accomplished, a plausible explanation could be offered why the blossoms were less susceptible to infection after being pollinated. A set of observations was therefore devised to settle this question. We felt warranted in concluding that nectar is produced in sufficient quantity to support the blight organism for 12 to 15 days after pollination; at least, a liquid resembling nectar remains moist in and about the nectar glands for that period.

What interpretation shall we give to the results obtained from our pollination and inoculation experiments? It is seen that in series A of these experiments, we obtained 100 percent of blight infection when the intervals between pollination and inoculation were 5, 24, and 49 hours, but when the intervals were increased to 72 and 96 hours no infection occurred; instead, 100 percent of fruit remained set on the trees. In series B, 100 percent of infection occurred when the interval was only 19 hours, but none developed when the time was extended to 48 hours or longer, while pollination continued to be effective even when the interval between castration and pollination was raised to 120 hours. Intervals higher than these gave neither infection nor fruit in this series, the blossoms having so lost vitality that they invariably dropped.

In series I, we obtained infection up to an interval of 144 hours, after which infection seemed impossible, and instead a high proportion of the fruit remained set on the trees. In series J, with the short interval of 24 hours between pollination and inoculation, 100 percent of the blossoms contracted blight, 67 percent when the interval was 48 hours, and 33 percent when the interval was 72 hours. There was no infection when a longer interval was used, but in the majority of cases 100 percent of the apples continued to develop normally on the trees. In series K, we obtained infection up to an interval of 144 hours between pollination and inoculation, but not longer. In series L, we obtained infection up to an interval of 72 hours but not longer; in series M, up to 120 hours but not longer; in series N, O and P, no infection and but little set of fruit, especially with P. The interval between emasculation and pollination was too long in these latter series, the result first becoming conspicuous in the series N, where this interval was 108 hours, and yet more emphatic when this was lengthened 24 hours more for series O, 48 hours more for P, and 72 hours more for Q.

We obtained blight infection in no case from our inoculations where more than 144 hours had elapsed after pollination, and there was disclosed no high probability of infection unless the organism was introduced into the blossoms within 72 hours or 3 days after pollination.

Are we justified in concluding that if pollination could be effected simultaneously with the opening of the blossoms, and that if all of these opened at the same time, the orchard would be proof against blossom blight 3 days later? It looks so. How can we most nearly approximate such hypothetical requirements? Doubtless the beekeeper and many orchardists will answer, "By having a host of bees ready to effect pollination as soon as the blossoms open". While our work needs to be confirmed on a larger scale than we have been able to carry it on, we can not now see why this answer is not correct. Yet we may eventually be compelled to conclude that the rate at which the bees effect pollination and the rate at which they carry infection varies so much according to fortuitous circumstances from year to year, that any general conclusion regarding their role as carriers or restricters of blight infection can hardly be reached at all.

We have watched the bees season after season, beginning activity with the opening of bloom, and making certain that a great number of blossoms would be young apples and past the possibility of infection 3 or 4 days later—long before any general blight wave could appear. We have seen this activity maintained with the same beneficent results until the middle of the blooming period and sometimes until near its end, when a withering wave of blight *seemed* to proclaim that the bees and the hive, the pollen they carried, and a host of other agents were all infected and the chance for any additional set of fruit was almost nil. In such a season, the bees could hardly appear as anything else than millions of friendly little sprites, starting out at the beginning of the season quickly to transmute the blossoms into blight-proof fruits, and keeping up the good work until the trees were laden to the danger point, when, presto! After a few showers and a day or two of good incubating weather for blight, the sprites would hasten from blossom to blossom as if to make sure that no more fruit should be permitted to further weight the trees. But if the showers come earlier, widespread infection may come earlier, and perhaps in some seasons, the blossoms are blighted off before a sufficient number are pollinated. Would not a great abundance of bees in the orchard in such a season improve matters by insuring the prompt pollination of every early blossom as soon as possible after it opens? We have never known an orchardist who keeps plenty of bees, that does not constantly and consistently secure as large or larger crops of fruit than his neighbor, who keeps no bees, provided that other things relating to the orchards are equal; and we have seen no more, if as much, blight in the orchards of bee-keepers as in those of non-keepers of bees.

It should be remembered that blossom-blight infection does not, as a rule, penetrate further than to the hardened fruit spurs; but since rains dash the organism from the blossoms over the branches and leaves inhabited by aphids and coated with honey-dew, where it can live; and, also, because both sucking and biting insects bite into blossoms and later into tender growing wood, we are obliged to conclude that much, but not all twig blight, can be traced back to blossom infection.

BLOSSOMS MAY FURNISH INITIAL INFECTION

In 1914, the senior writer planted out 20 pear trees of 10 different varieties. For several seasons he pinched off all blossom buds before they opened to enable the trees to get a good start before inviting the entrance of blight. So long as this practice was continued, not a sign of blight appeared at any time in this orchard, but when the war broke and timely attention to removing the blossoms in the spring of 1918 was neglected, conspicuous blossom blight developed which was perpetuated throughout the summer in the form of twig blight. A large part of the tops of several trees died out a few weeks after blossoming and these trees today are smaller than they were in 1918, and they blight to some extent every year. Also see p. 87.

SUCKING INSECTS AND TWIG BLIGHT

This phase of blight has been considered by many writers and is so well understood that we think it unnecessary to consider it at length. Sucking insects are its purveyors and several observers have noticed that it can be prevented to a considerable extent by killing off, early in the season, the aphids, plant bugs, leaf-hoppers and other pests which scatter it. Among the sucking insects which have been definitely proved to inoculate healthy twigs, by inserting into them their contaminated beaks, may be named the Tarnished Plant Bug (*Lygus pratensis*), *Campylomma verbasci, Orthotylus flavosparsus, Ilnacora malina, Adelphocoris rapidus, Empoasca mali, Eriosoma lanigera, Rhopalosiphum prunifolium,* *Anuraphis roseus, Aphis pomi.* Some other sucking insects have been quite definitely proved to be transmitters of the disease. The list named ought to satisfy all inquirers that any insect of sucking habit, which sinks its beak into an infected twig becomes a potential carrier and will almost certainly transmit the disease if the next twig it punctures is at a favorable stage of development.

In some seasons twig blight becomes disastrously prevalent without being preceded by much if any blossom blight, though this cannot be said to be the rule in Ohio. Such outbreaks are brought about by the conjunction of local exuding cankers in the orchard, rains to scatter the organism over the foliage, favorable temperatures for incubation and the presence of numerous sucking insects, generally those which excrete more or less honey-dew, such as aphids, the pear psylla and leaf-hoppers.

MISCELLANEOUS INSECT CARRIERS

Some boring insects, for example the fruit tree bark beetle, (*Scolytus rugulosus*) have been proved to be purveyors of blight. Insects ovipositing in tender twigs may scatter it both directly and indirectly. For instance, we encountered an orchard badly stung by buffalo tree-hoppers and suffering so excessively from blight that it was inferred that the insects had contributed to the spread of the disease. Whether this was done by feeding punctures made comparatively early in the season, or by punctures made by the ovipositor, these scars later becoming inhabited by infected woolly aphids, we could not determine. We met another case where the apple flea weevil, *Orchestes pallicornis,* was also quite certainly an important purveyor of blight. Any contaminated insect, visiting the blossoms while they are open may infect them, whether it be of the biting or sucking class. Thus flies, wasps, wild bees and plant bugs may become carriers, and ants which maintain close relations with all the aphids for the sake of their honey-dew, we must believe are important carriers.

Sometimes blight is seen on young trees in the nursery row which have never blossomed. In such cases, infection is generally accomplished in some such manner as just described. In one instance, we observed a clump of several seedlings in a nursery row with most of the branches blighted. They were several rods to the northeast of a large tree with much blight in its top. A little while before, several rain storms, driven by strong winds from the southwest, had occurred. Leaf-hoppers, aphids and mites probably completed the infection after the plants were bathed with infected water.

RELATION OF ANTS TO SPREAD OF FIRE BLIGHT

When we commenced this investigation, we conjectured that ants might become infected very early in the season from associating with woolly aphids which are so apt to hibernate in blight cankers around the collars of the trees. Some writers have supposed that the bodies of the ants are reservoirs for carrying the infection over winter. Both of the above suppositions may prove to be true, but we think in the light of all our investigations herein reported, that the rapid development of the scourge is easily explained in other and more probable ways.

In an attempt to prevent ants from initiating a scourge in our orchards, in the spring of 1916, we put bands of Thum's Tree Tanglefoot around all the trees in more than 40 acres of orchard, making no discrimination between apple, pear, plum, peach, cherry or other kinds of fruit. Banding was commenced April 8 and the last bands were put in place April 24. No activity by ants was noted until sometime after these dates. Many ants must hibernate in nests in the trunks of the trees or beneath the bark, for they were quite abundant all over the orchard in the tops of the trees by blossoming time. However, it was not possible for them to have become infected from woolly aphids which had wintered near the base of the trees. So far as could be discovered they did not influence in any way the coming of blossom blight. With one or two exceptions all cankers and blighted limbs had, so far as possible, been cut out and removed from the orchard; hence, they had practically no chance at all to become contaminated and initiate the scourge of blight which arrived toward the end of the blooming period. We suspect them to be important carriers of the disease, but do not believe they contribute greatly to its spread until it has gotten under good headway through other agencies.

ARE APHIDS, APHID WAX AND SYRPHID LARVAE POTENTIAL RESERVOIRS?

To ascertain whether the bodies of woolly aphids or the wax they deposit might be reservoirs in which the organism survived over winter, or was acquired in early spring, we captured living lice secreted in crevices in the bark around a living blight canker in early April, 1916, and tried to isolate the organism from their bodies on agar plate cultures. We did the same with the wax collected from the borders of cankers. While this work was not sufficiently extensive to raise a strong presumption against the bodies of lice and the wax serving as reservoirs, the results were wholly negative. Since syrphid larvae live on aphids and might become a storehouse for germs, we tried to isolate the organism from a hibernating larva taken from among woolly aphids around a blight canker. The results were negative.

DOES THE BLIGHT ORGANISM SURVIVE IN INTESTINES OF BEES?

We tried to answer this question by confining two bees in a large glass jar at 4:15 p. m. and giving them for food sterilized honey diluted with 50 percent sterile water which was infected with a pure culture of the blight organism. The bees were not noticed to feed until the next morning at 7:45. The honey was removed at 8:20 a. m. From 10:00 a. m. to 10:30 a. m. one bee was dissected, being first prepared as follows:

> We cut off the head, singed the body slightly over flame, sterilized body 1 minute in corrosive sublimate (1-1000), washed it in sterile water and dried it in sterile cotton. The entrails were then removed with sterile instruments, macerated and put into 25 cc. physiological salt solution and kept for 1 hour with frequent shaking of the flask.

Plate cultures were then made and organisms in any way resembling blight were inoculated into the growing tips of Northern Spy seedlings growing in a flat in the greenhouse. Ten different organisms were isolated and tested in this manner. Results were negative.

We decided that the possibility of the blight organism being taken into the intestinal tract of bees, surviving there and then being scattered in the excreta of the insects was so unlikely that further consideration was not warranted.

HOLDOVER CANKERS OF BLIGHT

Holdover cankers of blight are conceded to be the reservoirs of infection from which the disease is renewed every spring. It has been generally supposed that insects visit these cankers early in the season, become contaminated, and then scatter the blight to blossoms, leaves and twigs. However, the most persistent observers of these cankers in the early part of the season have always been puzzled by such claims, for no insects seem to seek these exuding cankers in early spring, though later, when some fermentation develops, they are visited freely by flies, wasps, etc. After watching closely for several seasons at Wooster, we affirm that very few cankers in this locality have become active by blossoming time, and that before the bloom only a few accidental insect visitors come in contact with them at all. In 1916 we could only locate three active cankers, before blooming, and we saw no insects at all visiting them. One canker was exuding April 7 and judging by the exudate must have become active by April 1; another was found shortly after April 7, and the third April 25. In 1915 we knew of three cankers, slightly active before blooming, and by watching for several hours per day on several different days saw two or three ants, an elaterid, and a lampyrid or two crossing over them in a perfectly normal manner, not stopping to feed nor giving the slightest hint that they were in any way interested in the area over which they traveled. We felt justified in doubting whether these cankers had any connection with the wave of blossom-blight which a little later swept over the entire orchard.

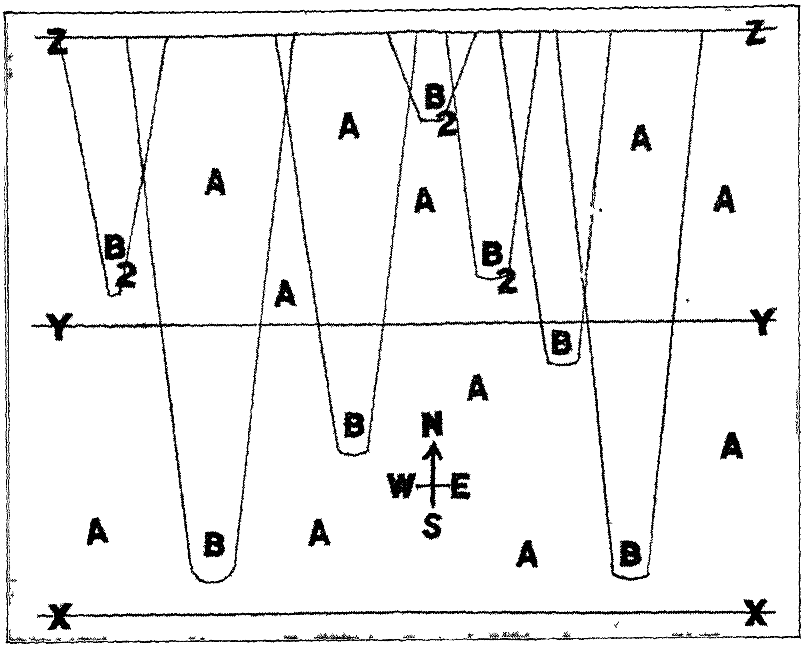
The beginning of spring infection comes, we believe, from rain washing over these exuding cankers, then dashing in greater dilution over open blossoms, over branches infested with aphids and smeared with honey-dew, also over colonies of psyllids and possibly over red bugs, tarnished plant bugs and like insects, which are able to complete inoculation and insure infection. However, when a whole orchard of 40 to 60 acres, exhibits blossom blight appearing suddenly over its whole extent, when only two or three or no holdover cankers at all could be found previous to the outbreak, one naturally concludes that the infection was carried into the orchard from an outside source and that very little of it can be justly ascribed to resident holdover cankers. These resident or local holdover cankers seem to us to signify much more, so far as blossom blight is concerned, to orchards considerably to the northward than to those in which they are found. They will probably be conceded by everyone to be capable of originating widespread twig and leaf blight which becomes visible at a later time, that is if an interval of a few weeks is given during which it can spread. We have seen them cause this phenomenon in seasons when practically no blossom blight was present, the infection getting under strong headway in June and July.

IS BLOSSOM BLIGHT A PROGRESSIVE WAVE NORTHWARD?

No one will question that a wave of blossom blight travels northward with the bloom—it must do this. But given an initial infection in some southern state, would a wave starting from this center be perpetuated northward from orchard to orchard and finally reach the Great Lakes if no other active cankers lay between the origin and the northern boundary? We do not know, neither does any one else. This seems not impossible, but improbable. But, if on the road northward, there are cankers active before or at the time of blossoming, say at the corners of each section, or even if not closer together than at the corners of each township, it seems to us that a wave of blossom blight can travel with a not very discontinuous, not to say, an unbroken front across several states from south to north, and that the infection of any particular blossom is likely in many cases to have come from a canker far to the southward, instead of from one a few miles or even 50 to the south. Of course, it must have been relayed from blossom to blossom, scores, possibly hundreds of times, on such a journey. At the same time, other blossoms, near by, possibly in the same cluster may owe their infection to a canker not more than 5, 10, or 50 miles to the southward, the infection having been relayed from blossom to blossom, or from blossom to tender twig all the way.

Since we have shown that other blossoms than apple and pear, such as peach, plum and cherry, may sustain the organism for several days, it may also be true that dandelion and most other or all honey-producing flowers do the same. If so, such a property has little or great significance in blight transmission, according to whether the organism merely survives without multiplication or propagates itself in this nectar. In the former case such flowers might not help greatly in bridging over wide gaps between orchards of apple and pear, but if there is sufficient multiplication to infect most or all of the nectar in the flower cups, the whole nectar sheet in all sorts of flowers furnishes a means by which infection can ride northward, despite long distances between orchards and active cankers. And here again we must take into account the possible influence of rain. If the organism has been deposited in the nectar, let us say of a violet or dandelion, will a little rain water falling into the flower cup distribute the organism all over the cup or through the flower head so as to render likely the contamination of all pollinating insects which visit the flower and, hence, multiply the chances for bridging over the gaps between orchards? More light on these questions is badly needed.

The following diagram may assist in understanding the relation of active and dormant cankers to the development and transmission of the disease as we conceive it possibly to be:



Let the points A be cankers which have not developed activity or exudate by blossoming time, and let the points B and B_2 represent cankers which have become active before apples and pears are in blossom. Let the lines X, Y, Z represent the isophanes which in this diagram indicate the centers of the zones of infloresence traveling northward.

When the north edge of the zone of infloresence is at the line X, there is no blossom blight, for no active cankers have been yet encountered. Of course, there can be no actual line X unless it be the southern coast line, but when the anterior edge of the zone is half way between X and Y and the center of the zone is at X, some blight will have developed about the centers B, if showers have occurred to carry the infection to the blossoms. Spread of the infection southward will eventually be cut off by lack of blossoms as soon as the zone of infloresence has passed over X, but northward, it can travel indefinitely with a constantly widening front from east to west, being relayed from blossom to blossom by insects and rain. When the center of the flowering zone has reached the line Y, the front may not have become continuous, but by the time it has reached the line Z the new centers B_2 will have so swelled the advancing front that it will be continuous. If the infection which started with cankers B, has become lost because of lack of carriers, such as insects and rain, the cankers B_2 will make good the gaps, and so the farther north the zone of infloresence travels the more certainly will there be a continuous blight wave, provided the conditions for transmission have averaged reasonably uniform and favorable from south to north.

The dormant centers A, which greatly out-number the active cankers B, B[1], etc. at blossoming time, later become active and are centers of infection for twig blight, which can spread about as far southward as northward, because the period of twig growth occupies a much longer time than blossoming, and tender twigs will develop to the southward as well as to the northward until late summer. Also during the passage of the wave of blossom blight, it is believed that some new small cankers are developed in spurs bearing blighted blossoms and on many twigs bearing sucking insects, the infection being carried to the latter by rain from blighted blossoms.

So long as insects were thought to be the only agents for transmitting blight, and it was further supposed that apple, pear and quince blossoms were practically the only ones concerned in supporting the organism, it taxed one's credulity considerably to be asked to believe that initial infection started many miles from the points where blight was observed to develop; but now that water has been proved to assist so greatly in its rapid development that scores or even hundreds of blossoms can be infected in a few moments from a single center which insects have established; and, further, that other blossoms than apple and pear may harbor the organism for several days, it becomes difficult to reject the logical deduction that infection will travel northward as a wave spread out over the zone of inflorescence, being limited and interfered with, of course, by such adverse factors as cold, drought, etc. Such a deduction seems hardly capable of direct proof, because it is impossible to follow infection from blossom to blossom or from orchard to orchard, even for a few miles, let alone over several states. Such a limiting factor as temperature may break or cause the disappearance of the wave in some instances.

The viewpoint which has hitherto been commonly presented rests wholly upon deduction; and the assumption underlying it, namely, that insects visit the exuding cankers before or at blossoming time, and then carry the infection over the whole orchard or neighborhood has been frequently questioned or denied by those who have observed most closely; and, so far as Ohio is concerned, we are satisfied that such insect visits are rare and accidental. We believe that the grounds from which the commonly accepted viewpoint is deduced, are neither better established nor more complete than those upon which our viewpoint is based.

EXPERIMENTS ON INSECT POLLINATION

To determine the influence of insect pollination on productivity of apples, we made the following experiments:

On May 11, 1916, we selected a late-blooming tree and pinched from three different branches all buds that were sufficiently open to permit of pollination by insects, counted the unopened flower buds remaining, and then enclosed the branches in cheesecloth bags to insure the exclusion of insect pollinators. On the same day we pinched from three other branches all open blossoms and counted the flower buds remaining. These branches were marked for future identification, but were not bagged to exclude insects from the flowers. On June 26, we counted the fruits set on all of these branches. The results are exhibited in the following table:

Covered branches				Uncovered branches			
Branch No.	Buds	Fruits	Percentage of fruit to buds	Branch No.	Buds	Fruits	Percentage of fruit to buds
1	177	2	1.13	1	62	6	9.677
2	240	5	2.083	2	183	36	19.672
3	228	2	.877	3	72	19	26.388
Average percentage of fruit to buds.			1.363	....	....	....	18.579

We have previously referred to an apple tree of the Wealthy variety (p. 87) which was covered over completely with a tent of cheesecloth before it bloomed. When in blossom a hive of bees was put under it and it was inoculated with blight. This tree was larger than either of two uncovered trees standing beside it. Of course, the covered tree lost much of its crop through blighting of the bloom, but lack of cross-pollination also seems to have been a factor in reducing its crop. The two uncovered trees also blighted considerably toward the end of the blooming period. The yield records of the three trees were as follows:

Uncovered	Bushels, firsts	Bushels, culls	Total bushels
Tree number 1	5.3	1.5	6.8
Tree number 2	4.2	1.3	5.5
Covered tree..................	3.3	1.7	5.0

Most of the apples on the covered tree had only a few seeds, averaging about 2.5 seeds per apple. Lack of cross-pollination is indicated by the few seeds per fruit. Since bees were abundant on the tree it must have been abundantly self-pollinated. The development of the pulp was not affected by the dearth of seeds.

CONTROL MEASURES

Experimental work on control measures for fire blight was carried on independently by the Department of Botany and only one experiment was performed as part of the cooperative program. This was with the electric violet ray which is reputed to possess germicidal properties.

EFFECT OF ELECTRIC VIOLET RAY ON FIRE BLIGHT

The machine used was Roger's Violet Ray High Frequency Generator, type H. The voltage used was 120.

Three small potted apple trees with succulent growth were used. No. 1 was inoculated with blight and then treated with the violet ray. No. 2 was inoculated, but not treated with the violet ray. No. 3 was not inoculated but was treated with the violet ray. The five twigs on No. 1 all contracted blight and exhibited exudation drops 4 days later, proving that the violet ray did not destroy the blight organism. No. 2 had the two inoculated twigs exuding blight 4 days after inoculation. No. 3 showed some injury to leaf-tips from the violet ray. We decided the violet ray was valueless as a treatment for blight.

TREATMENT OF LOCAL CANKERS AND OF SUCKING INSECTS

Because of the relationship indicated by these researches between exuding cankers, blighted blossoms, rain, insect honey dew and sucking insects, the recommendations which have quite generally been made by other writers for the destruction, early in the season, of local cankers and sucking bugs must be emphasized even more than formerly.

The most effective remedy thus far found for killing sucking insects is to spray with a nicotine-soap solution or in some cases apply a nicotine dust with a dusting machine.

SHALL THE ORCHARDIST ENCOURAGE OR DISCOURAGE THE KEEPING OF BEES?

The writers have not been in doubt regarding the answer that should be given to this question. The adaptations of bee to blossom and blossom to bee are of age-long development and perfect or satisfactory pollination cannot be expected if abundant pollinators are not present. The experiments herein recorded (see p. 121) indicate failure to set fruit when insects are excluded and a short crop of well-nigh seedless fruits was produced (see p. 122) when cross-pollination was prevented. Regular crops of fruit from an orchard that suffers or even dies from blight after several years, are better than meager, irregular crops from an orchard that never suffers seriously from blight. But fortunately, bees do not compel the orchardist to choose either horn of such a dilemma; there would apparently be some disastrous blight years if no honey bees were in existence, and from our researches (p. 108) we believe it is fair to infer that in the early part of the blooming season bees do not scatter much blight, but by promptly pollinating the blossoms as fast as the stigmas ripen, hurry such fruit past the period of susceptibility to blight, so that in about 3 days after pollination, such blossoms or fruits will scarcely blight at all. This explains why it is that orchardists who also keep numerous stands of bees have full crops of fruit, even when blossom blight is very bad. While it seems to be true that bees are among the most effective disseminators of blossom-blight toward the end of the blossoming period, this may in large measure be condoned or in some cases regarded as advantageous, since their work in killing the blossoms will reduce the work of thinning, an operation that may be necessary if too many fruits have set. If it is also true, as we have conjectured, that a wave of blossom blight advancing northward from the south plays a larger part in producing blight epidemics than has been hitherto admitted, it seems obvious that numerous bees in the orchard to keep pace with the earliest blossoms will be of much assistance in producing fruits instead of blighted blossoms. If this conclusion is true, a great consideration will be to get as much pollinating as possible accomplished before the blight wave arrives.

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SUMMARY

1. Our investigations have proved that the organism of fire blight may live in honey for 72 hours or more, but this approaches the optimum limit.

2. We found that fire blight developed in tender twigs that were inoculated with honey drawn from three different hives, two of which were normally located. From two of the twigs thus blighting, the organism was recovered and by reinoculation into other twigs was proved to be fire blight.

3. By using apple pollen removed from the baskets of bees caught as they were entering the hive to inoculate into tender twigs, we obtained several cases of death, apparently from fire blight, but the organism was not recovered nor its identity proved.

4. By inserting the mouthparts of bees, caught during blossoming, when entering their hives, into incisions into tender twigs, we obtained some twigs which seemed to have died without doubt from fire blight, but the organism was not recovered nor its identity proved.

5. The proof for Nos. 2, 3 and 4 is much strengthened by noting that the results for all three projects were obtained on the same dates, May 23 and May 24.

6. The fire-blight organism was proved to be able to live in aphid honey-dew for 7 full days until the dew was completely dried and then for 3 more days or perhaps longer when moisture was added.

7. The organism was also proved to be able to live in peach, plum, and cherry nectar for 5 full days or probably more and capable of producing blight in apple twigs when inoculated into them.

8. Rain was proved to be a most important carrier of infection over a tree after centers were established, especially if near the top. We estimated that from 50 to 90 percent of all blossom infection is accomplished by infected rain water.

9. We proved that blossoms which have been pollinated for 72 hours are not likely to be inoculated with fire blight and that susceptibility to inoculation does not exist in blossoms that have been pollinated for 144 hours.

10. We infer from number 9, that numerous honey bees in an orchard to fertilize the blossoms as soon as they open will insure a crop of fruit by putting the early blossoms past susceptibility to blight before the disease is generally diffused over the orchard.

11. The initial infection of a young pear orchard was observed in one case to have originated through blossom blight.

12. Several sucking and boring insects were observed to be carriers of fire blight.

13. Efforts to prove that ants were responsible in a large way for early spring infection met with negative results.

14. Attempts to isolate the blight organism in early spring from the bodies of aphids, aphid wax and syrphid larvae taken from living blight cankers met with negative results.

15. We submit that there is ample ground for crediting blossom fire blight with traveling northward with the zone of inflorescence to a greater extent than is now accepted.

16. Branches covered with cheesecloth bags to exclude insect pollinators set fruit for only 1.36 percent of the blossoms while 18.58 percent of the blossoms set fruit on uncovered branches.

17. The application of the electric violet ray to leaves and twigs to test its killing power on the fire-blight disease gave negative results.